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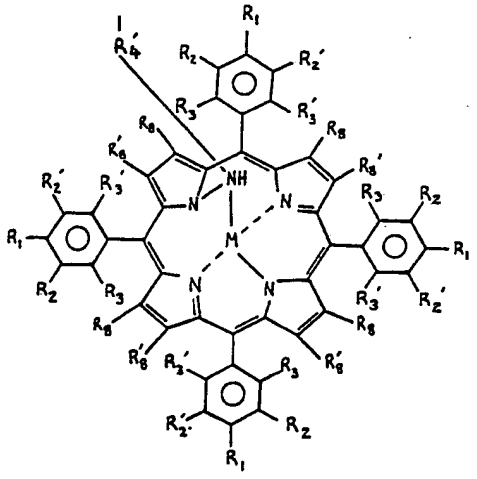
(54) Title: TETRAPHENYLPORPHYN DERIVATIVES

(57) Abstract

A hapten formed of a metalloporphyrin cofactor bound to a residue of a substrate. The hapten is designed to mimic a transition state of a metalloporphyrin catalyst and the substrate in a reaction and is characterised in that the metalloporphyrin cofactor is of formula (II), in which: R_1, R_2, $R_{2'}$, R_3, $R_{3'}$, R_8 and $R_{8'}$ are as defined in the specification; R_4 is a bridging group connecting the metalloporphyrin catalyst to the residue of the substrate; and M is a metal ion having a co-ordination number of at least 4. Also claimed are metalloporphyrin cofactors in which R_4 is H, a linker group or a removable protecting group.



(II)

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TETRAPHENYLPORPHYN DERIVATIVES

This invention relates generally to catalytic, antibody-controlled processes in which tetraphenylporphyrin catalysts are used. The invention also relates to tetraphenylporphyrin derivatives, haptens containing them, antigens containing the haptens, and antibodies raised to the antigens, which have application in the processes.

Metalloporphyrins are able to act as catalysts for many types of chemical reactions; especially oxidative transformations such as hydroxylation reactions, dealkylation reactions, epoxidation reactions, desaturation reactions and the like (for example, Dixon, M. and Webb, E.C.; Enzymes, 3rd Ed.; Academic Press, 1979; Collins, J.R. et al; 1991; J. Am. Chem. Soc., 113, 2736-2743 and Rettie, A.E. et al; 1988; J. Biol. Chem., 263, 13733-13738). However the selectivity of chemo- and/or regio-selective attack (or oxidation) of metalloporphyrins is usually poor and cannot be predetermined for any given substrate. Usually when a metalloporphyrin catalyst is used, a complex mixture of isomeric and non-isomeric products will be obtained, which are difficult to separate.

To control product formation, it has been recently proposed to use metalloporphyrins as catalysts or cofactors in antibody mediated reactions; the antibody bringing site-selectivity to the process (Schwabacher, A.W., Weinhouse, M. I., Auditor, M.M and Lerner, R.A.; 1989; J. Am. Chem. Soc., 111, 2344 to 2346). The authors' proposal is to immunise an animal with a complex of a substrate and a metalloporphyrin chosen to bind to the substrate. Antibodies having binding sites that are complementary to both the porphyrin and the substrate are then isolated. It is then proposed to bind a porphyrin catalyst to such an antibody so that only substrate that is correctly orientated can be bound and reacted.

Schwabacher et al managed to prepare antibodies to Fe^{3+} and Co^{3+} complexes of synthetic meso-tetra-kis(4-carboxyphenyl)porphine by coupling the complexes to keyhole

limpet hemocyanin (KLH) or bovine serum albumin (BSA) and applying standard monoclonal techniques. However no further steps of the proposed process were carried out.

EP 0305870 discloses a similar concept, in general terms, in which an immunoproximity catalyst for chemical reactions is prepared by selecting a hapten which corresponds to, but is different from, a transition state complex of the reactant and a catalyst. An immune response is then stimulated using an antigen derived from the hapten to produce antibodies to the hapten. The antibodies are then isolated. "Converting" haptens are then used to covalently bind the catalyst to the antibodies to produce "modified" antibodies. The modified antibodies are then isolated for use. These modified antibodies catalyze cleavage of bonds in the target molecules; much in the manner of an enzyme. The antibodies are said to speed up the reaction and to introduce site-specificity.

EP 0305870 suggests that the catalysts could be general acid-base catalysts, nucleophilic catalysts, electrophilic catalysts and metal catalysts. No specific mention is made of metalloporphyrins. Also, for many processes, the isolation of a transition state complex for many of these catalysts may well prove to be difficult, if not impossible. In any event, EP 0305870 does not disclose any specific process examples which illustrate that the modified antibodies had in fact been prepared.

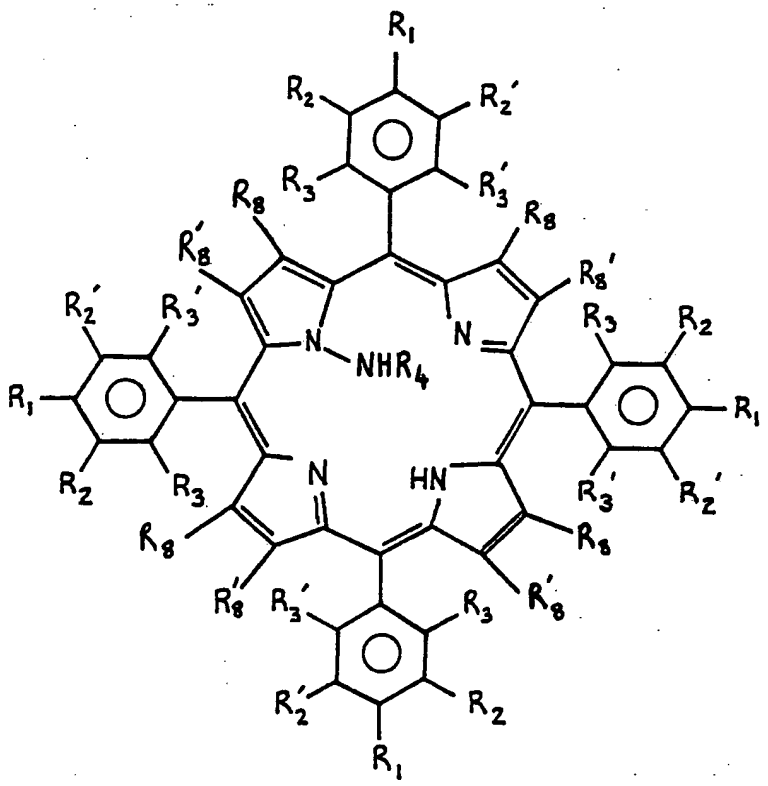
PCT patent publication WO 92/01781 discloses a similar process in which metalloporphyrin derivatives are used as cofactors or catalysts. Also proposed in general terms are porphyrins derivativised with alkyl groups so that the resultant antibody would have alkyl or aryl binding sites. The derivatives are used to generate haptens that mimic the actual catalyst and the substrate in the relative orientation and spacing needed for the reaction to proceed. The haptens are then used to generate antibodies which have binding sites complementary to the catalyst and the substrate in the correct orientation. Unlike the process

described in EP 0305870, the antibodies need not have the catalyst covalently bound to them prior to use.

Unfortunately it is not a simple matter to create haptens from the metalloporphyrin derivatives disclosed in the PCT publication. This is because coupling of a substrate to the porphyrin is not possible because there are no convenient points of attachment on the porphyrin.

Accordingly it is an object of this invention to provide a metalloporphyrin derivative that can be readily attached to a substrate to provide a hapten. It is also an object to provide haptens containing the metalloporphyrins, antigens containing the haptens, antibodies raised to the antigens, processes using the antibodies, and catalysts for use in the processes.

In one aspect this invention provides a compound of the formula I:



I

in which:

each R_1 is independently selected from -H, -F, -Cl, -Br, $-CH_3$, -COOH, $-SO_3H$, $-COO-C_{1-6}$-alkyl, -CH=CH-COOH,

$-CH=CH-COO-C_{1-6}$-alkyl, $-SO_3-C_{1-6}$-alkyl, $-NO_2$, phenyl, $-NH_2$, and $-NH-CO-C_{1-6}$-alkyl;

each R_2 and R_2' is independently selected from -H, -F, -Cl, -Br, $-CH_3$, -COOH, $-SO_3H$, $-NO_2$ and phenyl;

each R_3 and R_3' is independently selected from -H, -F, -Cl, -Br, $-CH_3$, $-O-C_{1-6}$-alkyl, $-NO_2$, phenyl, $-NH_2$, and $-NH-CO-C_{1-6}$-alkyl, or

at least one R_3 or R_3' is independently selected from (a) -NH-CO-alkylene-3N-imidazole or -NH-CO-alkylene-3-pyridine, in which each alkylene has 2 to 4 carbon atoms; and (b) -CO-alkylene-3N-imidazole or -CO-alkylene-3-pyridine, in which each alkylene has 3 or 4 carbon atoms; or

a pair of R_3 and R_3', on opposing phenyl groups, jointly form (c) -NH-CO-alkylene-3-pyridyl-5-alkylene-CO-NH- or -CO-alkylene-3-pyridyl-5-alkylene-CO-, in which each alkylene has 2 to 4 carbon atoms;

R_4 is a) a hydrogen atom; b) a linker group containing a reactive centre or group through which the compound of Formula I may be bonded to another compound; or c) a removable protecting group;

each R_8 and R_8' is independently -H, -F, -Cl, -Br or -CN; and

acid addition salts of the compound and sodium, potassium and calcium salts of the compound.

When R_4 is a linker group b), it is preferably of the formula $-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-A$ in which R_5 is $-(CO)-$, $-(SO_2)-$ or $-(POOH)-$, R_6 is -O-, -S-, or $-(NH)-$, each of m, n and p independently is 0 or 1 and A is a reactive leaving group or centre or, when p is 1, A may also be a hydrogen atom.

More preferably m, n and p are 0 and A is halogen, particularly Cl or Br. A particularly preferred linker group is -COCl, which may easily react with a functional group such as -OH or $-NH_2$ on another molecule to form the bridging group -CO-O- or -CO-NH-.

When R_4 is a removable protecting group c), it is

preferably a protecting group which will protect the >N-NH_2 group against oxidation by a reagent such as 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), and which is removable by hydrolysis under acid or alkaline conditions. A preferred protecting group is CF_3-CO-, which may be removed by mild alkaline hydrolysis.

Preferably R_1 is H, a group which increases the water solubility of the compound, or a functional group which permits the attachment of a carrier protein to the compound. More preferably, at least one R_1 is -NH-CO-CH_2-CH_2-COOH or NH_2, at least one further R_1 is -COOH, -$COOCH_3$ or -CH=CH-COOH and the remainder are H.

Preferably R_2 and R_2' are all H. Preferably one R_3 or R_3', or one pair of R_3 and R_3' are selected from groups a), b) and c) as defined above for R_3 and R_3'. The remaining R_3 and R_3' are preferably H. R_8 and R_8' are preferably H.

Compounds of formula I in which R_4 is m-$CH_3C_6H_4SO_2$- and $O_2NC_6H_4CO$- are known, but these two groups are neither linker groups since they have no reactive atoms or groups for binding, nor are they protecting groups which can be removed without destroying the >N-N< bond.

The C_{1-6}-alkyl may be any branched or unbranched alkyl group that contains up to 6 carbon atoms. Methyl is preferred.

The use of N-amino porphyrin compounds greatly facilitates the synthesis of haptens since a linker group can be readily attached to the nitrogen atom that has been added. Then a desired substrate or a functionalized derivative of a desired substrate may be attached to the linker group.

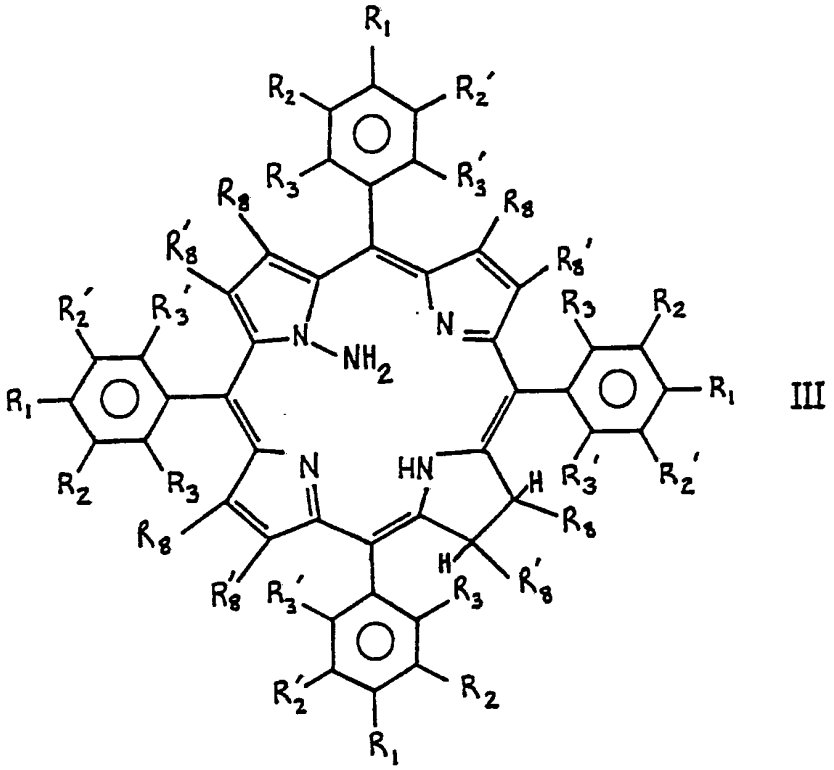
The invention also provides a compound of formula I, as defined above, for use in the preparation of a hapten that mimics a transition state of a metalloporphyrin catalyst and a substrate in a reaction.

The invention also provides a process for the preparation of a compound of formula I, as defined above, comprising the steps of:

a) for a compound of formula I in which R_4 is H, deprotecting a compound of formula I in which R_4 is a protecting group;

b) for a compound of formula I in which R_4 is a linker group, reacting a compound of formula I in which R_4 is hydrogen with a precursor of the linker group that contains two reactive centres or groups, one of which is capable of forming a bond with the $>N-NH_2$ group;

c) for a compound of formula I in which R_4 is a protecting group, i) protecting the $>N-NH_2$ group of a compound of formula III



III

in which R_1, R_2, R_2', R_3, R_3', R_8 and R_8' are as defined above; and ii) oxidizing the compound of formula III to the corresponding compound of formula I.

In step a) the deprotection step will depend upon the nature of the protecting group, but is preferably carried out by acid or alkaline hydrolysis. Where the protecting group is CF_3CO-, mild alkaline hydrolysis may be used; for example using EtOH/KOH or EtOH/$Ca(OH)_2$ at temperatures in

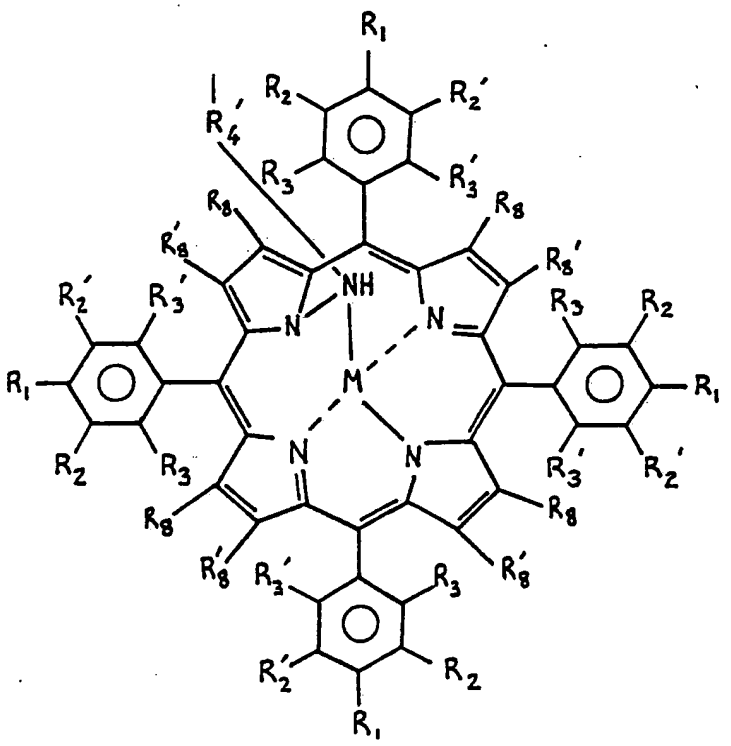
the 65°C to 75°C.

In step b) the precursor of the linker group may be, for example, $X-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-A$ in which X is a reactive leaving group or atom, preferably Cl or Br, and the other symbols are as defined above. Where the linker group is -CO-Cl-, a suitable precursor is phosgene or diphosgene.

In step c), where the protecting group is CF_3-CO-, the compound of formula III in which R_4 is hydrogen may be reacted with trifluoracetic anhydride in a polar non-aqueous solvent. The oxidation step ii) may be carried out using an oxidizing agent such as DDQ in an inert solvent, for example methylene chloride.

The starting material of formula III may be prepared by reacting the corresponding porphyrin with O-m-toluene-sulphonylhydroxylamine as described in Callot, H.J.; 1979; Tetrahedron, 35, 1455-6.

In another aspect this invention provides a hapten comprising a metalloporphyrin cofactor bound to a residue of a substrate, the hapten mimicking a transition state of a metalloporphyrin catalyst and the substrate in a reaction, and in which the metalloporphyrin cofactor is of the formula II:



II

in which:

R_1, R_2, R_2', R_3, R_3', R_8, and R_8' are as defined above for formula I;

R_4' is a bridging group connecting the metalloporphyrin catalyst to the residue of the substrate; and

M is a metal ion having a co-ordination number of at least 4;

or an acid addition salt thereof or a sodium, potassium or calcium salt thereof.

The bridging group may be any suitable bridging group, with the proviso that it should be selected such that the spacial orientation of the metalloporphyrin cofactor with respect to the residue is as close as possible to that of the transition state formed by the corresponding metalloporphyrin catalyst and the substrate during reaction. Once the transition state has been identified and the residue of the substrate selected, selection of the bridging group will be routine.

Preferably the bridging group is of the formula $-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-$ in which R_5 is $-(CO)-$, $-(SO_2)-$ or $-(POOH)-$, R_6 is $-O-$, $-S-$, or $-(NH)-$, m is 0 or 1, n is 0 or 1 and p is 0 or 1. Preferably m, n and p are 0. In a specific example the bridging group is $-(SO_2)-O-$ or $-C(=O)-O-$.

It will be appreciated that the hapten has the advantage that bridging group projects axially from the centrally located amino group. Therefore the hapten can more closely mimic the relative positions of the corresponding substrate and metalloporphyrin catalyst in the transition state.

When at least one R_3 or R_3' is independently selected from (a) -NH-CO-alkylene-3N-imidazole or -NH-CO-alkylene-3-pyridine, in which each alkylene has 2 to 4 carbon atoms; and (b) -CO-alkylene-3N-imidazole or -CO-alkylene-3-pyridine, in which each alkylene has 3 or 4 carbon atoms; or a pair of R_3 and R_3' on opposing phenyl groups jointly form (c) -NH-CO-alkylene-3-pyridyl-5-alkylene-CO-NH- or

-CO-alkylene-3-pyridyl-5-alkylene-CO-, in which each alkylene has 2 to 4 carbon atoms; a nitrogen atom in the heterocyclic ring acts as a fifth ligand for the metal ion M. The side of the porphyrin that has the fifth ligand is shielded and cannot come into contact with antibodies raised to antigens containing the hapten.

The substrate may be any molecule upon which an oxidative transformation, such as a hydroxylation, dealkylation, epoxidation or desaturation reaction, is to be performed. The residue is a group that corresponds to the substrate molecule and is bound to the bridging group. The residue may differ from the substrate in that it may contain an additional functional group through which it is bound to the bridging group. Alternatively the residue may bind to the bridging group through an atom or functional group existing in the substrate. In either case, the residue is attached to the bridging group in such a way to mimic a transition state of the substrate in a reaction pathway with a metalloporphyrin catalyst.

For example, the substrate may contain a non-activated primary, secondary or tertiary carbon atom which is to be hydroxylated. The residue would then comprise the substrate molecule with a hydrogen removed from the carbon atom or with the hydrogen replaced by a functional group that is bonded to the bridging group. In a specific example, in the preparation of Ser^{8}-cyclosporin A from cyclosporin A (CsA), the substrate would be cyclosporin A and the residue would be Ser^{8}-cyclosporin A bonded to the bridging group though the OH of Ser^{8}. The -O- of the hydroxy may be considered to be part of the residue or the bridging group.

As an alternative example, the residue may contain a group of the formula >N-alkylene'- in which the alkylene' may be any branched, unbranched, substituted or unsubstituted alkylene radical. In this case, the substrate will be a group of the formula >N-alkyl.

A specific example of such a substrate would be cyclosporin A in which the N-methyl of Leu^{4} is to be

hydroxylated to give N-hydroxymethylleucine[4].

In another example, the residue may contain a group of the formula -O-alkylene'- in which alkylene' is as defined above. The substrate would then have a group of the formula -O-alkyl and the hapten would mimic a transition state in the dealkylation and hydroxylation of the -O-.

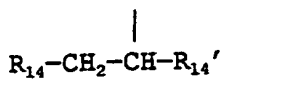
A specific example of such a substrate would be ascomycin (which is described in EP 184 162) in which -O-alkyl corresponds to the methoxy group on the carbon atom numbered 15. The residue would then be ascomycin but with one of the hydrogen atoms of the methoxy group replaced by a bond to the bridging group. The hapten would then mimic a transition state in the replacement of an alkoxy group with a hydroxy group on carbon atom 15 of ascomycin.

In another example, the residue may contain an aromatic group of which a carbon atom is attached to the bridging group. The substrate would then also contain an aromatic group and the hapten would mimic a transition state in the hydroxylation of the aromatic ring.

In another example, the residue may contain the group of the formula

(in which R_{12} and R_{13} are each independently a substituted carbon atom) which mimics an epoxy ring. In this case the substrate would contain the group $R_{12}=R_{13}$ which is to be epoxidated.

In a yet further example, the residue may contain the group



in which R_{14} is H or an unsubstituted or substituted alkyl

group and R_{14}' is an unsubstituted or substituted alkyl group. The substrate would then contain a group $R_{14}-CH_2-CH_2-R_{14}'$ of which the single bond between the CH_2-CH_2 is to be desaturated. Plainly the substituents on the groups R_{14} and R_{14}' must permit the desaturation of the C-C bond and hence the removal of a hydrogen atom from one of the carbon atoms. Specific examples would be the desaturation of dihydro-MeBmt[1] cyclosporin A to cyclosporin A and the desaturation of valproic acid to 4,5-dehydro-valproic acid.

In one preferred example, each of R_2, R_2', R_3 and R_3' is H and a least one R_1 is $-NH-CO-CH_2-CH_2-COOH$ or NH_2 and the others are H. The use of a hapten in which one R_1 is $-NH-CO-CH_2-CH_2-COOH$ or NH_2 facilitates coupling of a carrier protein to the hapten. Also the solubility of the hapten can be increased. The solubility of the hapten can also be increased by substituting the para-position of the phenyl groups with carboxy or ester groups.

Preferably the metal ion M is such that when it is coordinated in the hapten, it is inert; particularly to oxygen. For example, the metal ion may be Ni^{2+}, Zn^{2+} or Sn^{4+}.

In a further aspect, the invention provides an antigen comprising a hapten, as defined above, coupled to a carrier protein that is capable of causing an immunogenic response.

The carrier protein may be connected to the porphyrin portion of the hapten; especially to one of the R_1 groups. Alternatively the carrier protein may be connected to the residue portion of the hapten. The carrier protein may be any suitable protein such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or ovalbumin.

In another aspect this invention provides an antibody, or a fragment thereof, that binds to a hapten as defined above. Preferably the antibody is produced by monoclonal techniques. The antibody, or fragment, has the advantage that it has two binding pockets; one for the porphyrin portion and the other for the residue portion.

In another aspect this invention provides a process for the production of antibodies suitable for controlling

reactions in which a substrate undergoes reaction in the presence of a metalloporphyrin catalyst to give rise to specific regioisomers or enantiomeric pure compounds, the process comprising:

providing a hapten as defined above that corresponds to a transition state of the substrate and catalyst;

stimulating an immune response in a mammal, preferably a mouse, for the production of antibodies to the hapten; and

isolating and purifying those antibodies from the immune response that are specific for the hapten.

Preferably the antibodies are monoclonal antibodies.

The process may further comprise the step of selecting the antibodies by binding them to haptens as defined above that have been immobilised in chromatography columns or bound to tracer proteins.

In another aspect this invention provides a process for the oxidation of a substrate, in the presence of a metalloporphyrin catalyst, to produce a specific regioisomer or enantiomer; the process comprising:

providing an antibody as defined above that is specific for a hapten that mimics a transition state of the substrate and the catalyst;

providing a metalloporphyrin catalyst that binds to the antibody,

providing an oxidizing agent, and

combining the antibody, catalyst, oxidizing agent and substrate to permit the substrate to react.

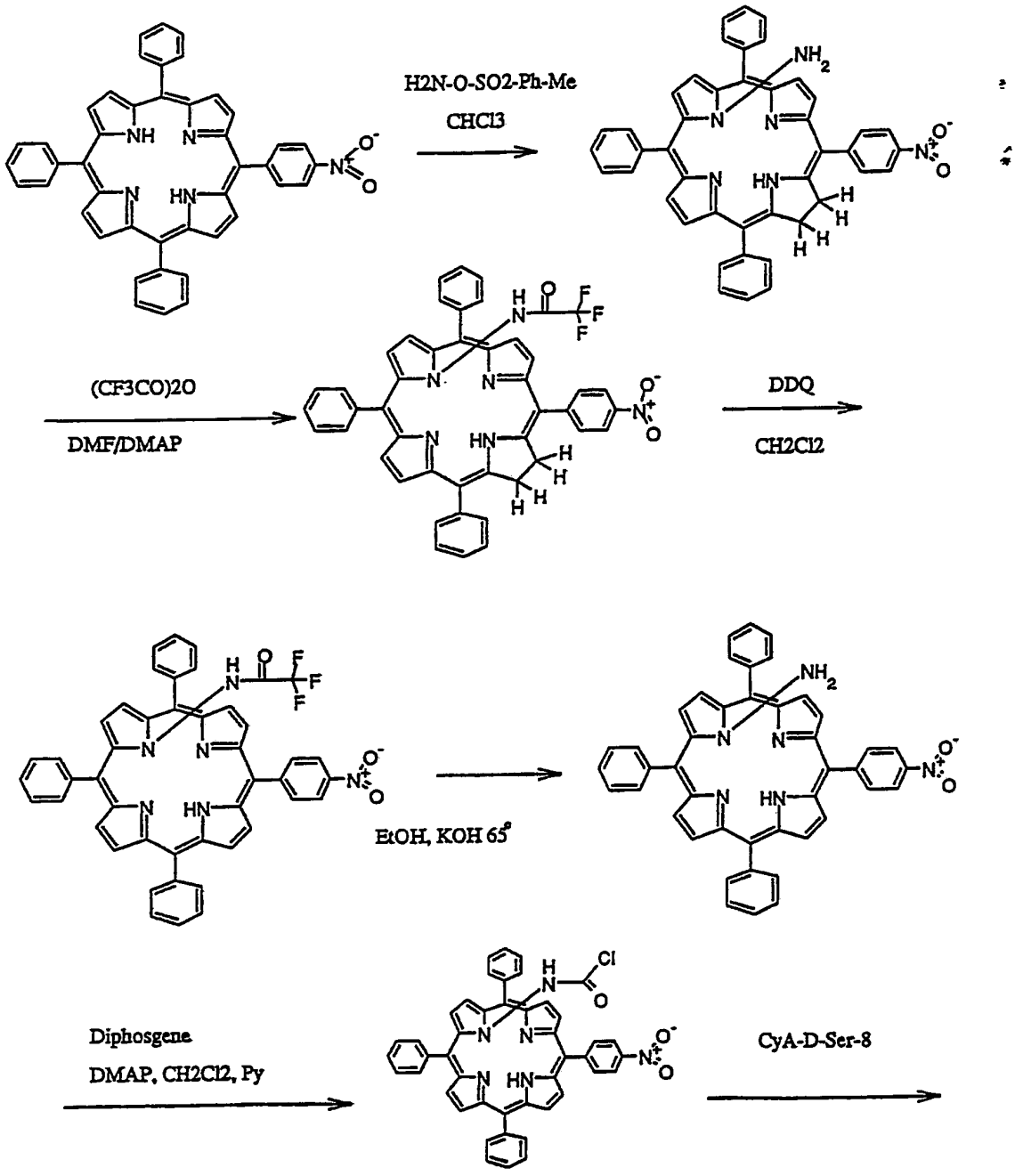
Preferably the metalloporphyrin catalyst is coordinated with a metal ion selected from Fe^{3+}, Cr^{3+} and Mn^{3+}.

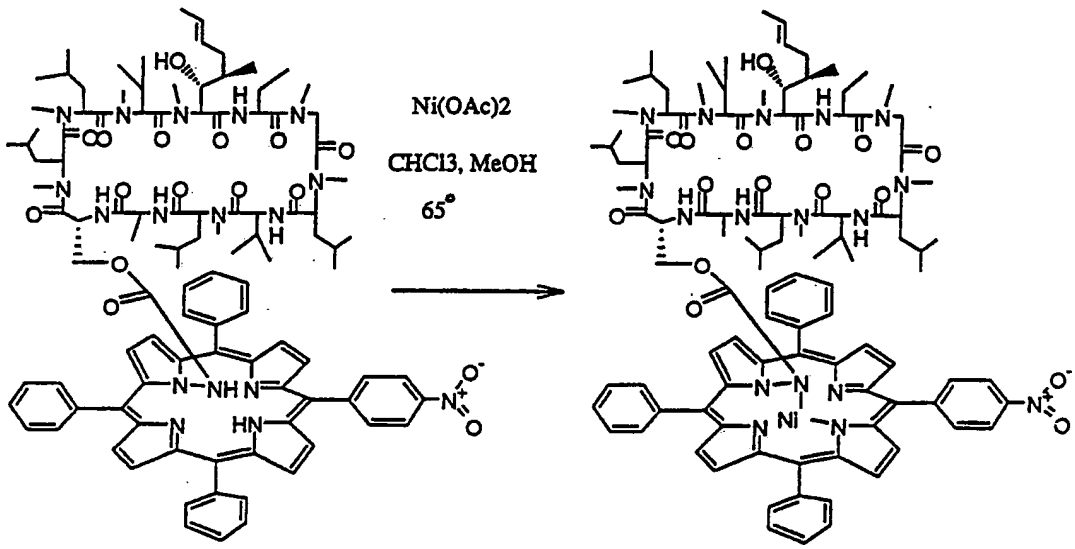
The N-amino-porphyrins of formula I may be synthesised by reacting tetraphenylporphyrin with O-m-toluenesulfonyl-hydroxylamine in a suitable solvent such as chloroform to produce N-aminotetraphenylchlorin. The N-aminotetra-phenylchlorin may be isolated and purified using chromatography. A suitable protecting group, for example a trifluoroacetyl group, may then be introduced to protect the introduced amino group and the compound oxidized to give N-

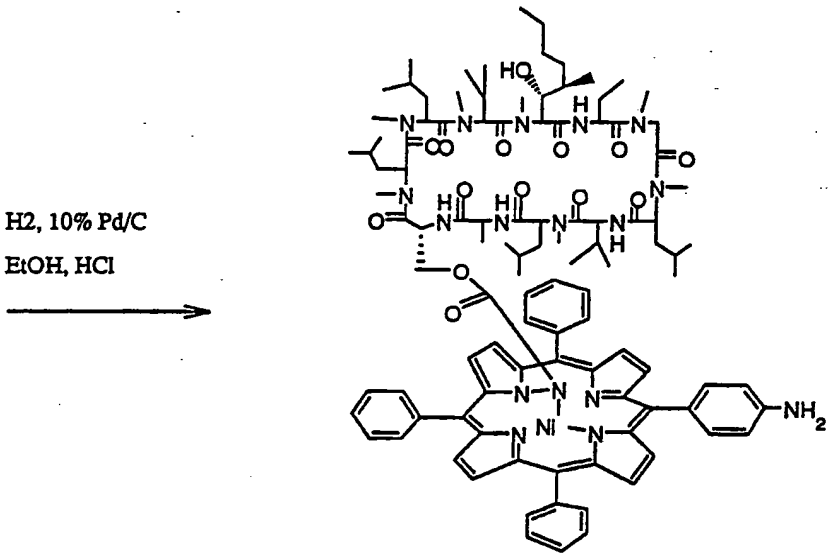
(protecting group)amino-tetraphenylporphyrin. The protecting group may then be removed and a suitable linker or bridging group added. A similar procedure is described in Callot, H.J.; 1979; Tetrahedron, **35**, 1455-6 in which N-tosyl-aminotetraporphyrin is produced. Callot did not use a removable protecting group and hence did not obtain amino-tetraphenylporphyrin, but the procedure described can be readily adapted. Methods of manufacturing porphyrins with a fifth ligand are known; for example Meunier et al; 1988; Inorg. Chem., **27**, 161.

The residue of the substrate may be produced by first synthesising or providing the desired product (ie, the substrate when reacted). This may be done using classical chemical pathways or by direct hydroxylation using a porphyrin catalyst. For example, N-hydroxymethylleucine^4-CsA may be produced by reacting CsA over a porphyrin catalyst in the presence of magnesium monoperoxyphthalate. The product is then covalently bonded to the bridging group of the aminoporphyrin, for example by condensation. The procedure adopted will depend upon the desired product but will be facilitated by the amination of the porphyrin. The adduct formed in the condensation step may be isolated and purified using chromatography.

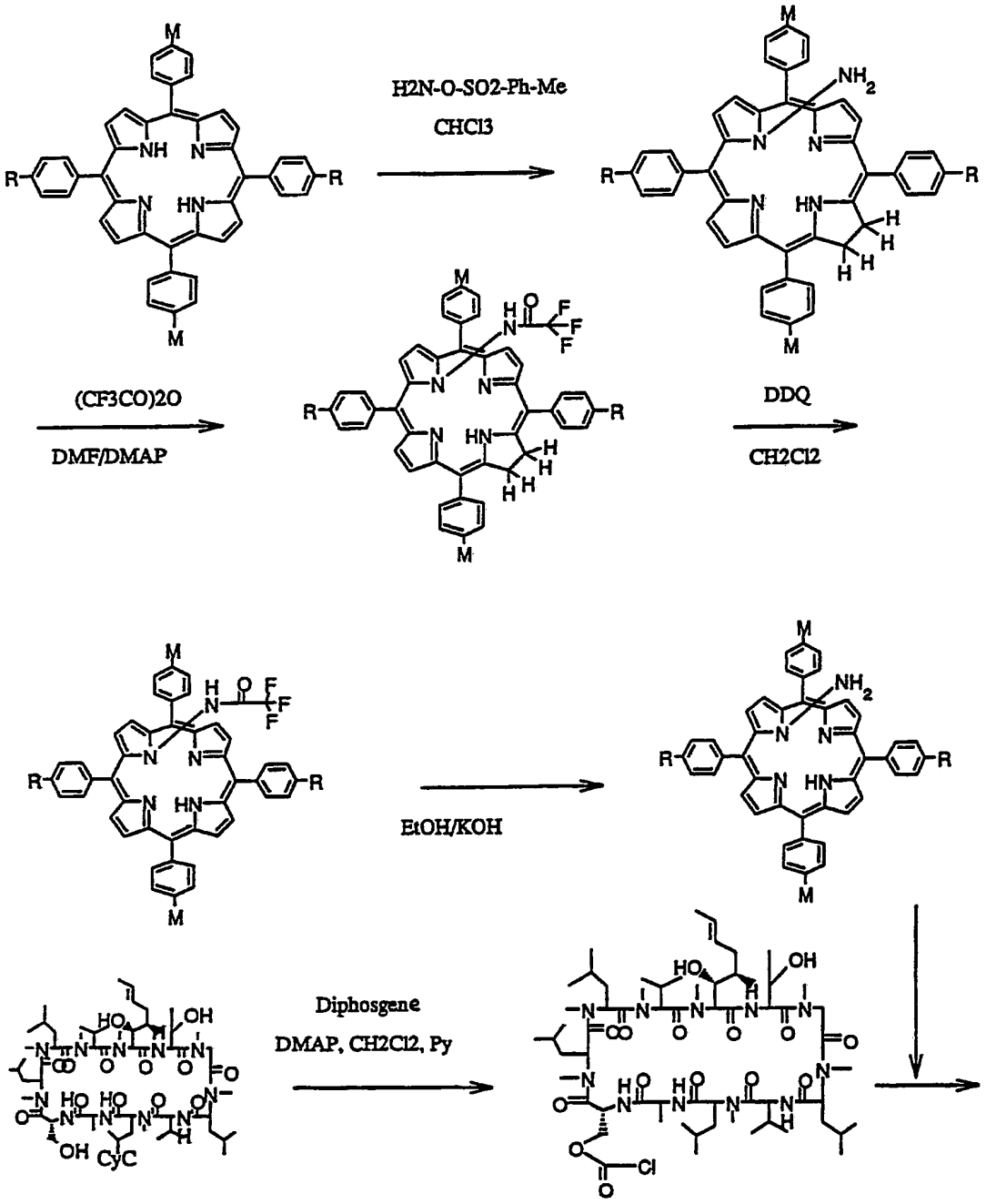
The adduct is then complexed with a suitable metal ion, for example by dissolving a salt of the metal ion in a suitable solvent and refluxing with the adduct. The metal ion coordinates between the introduced nitrogen and the three pyrrol nitrogen atoms of the porphyrin. The reaction scheme for the production of a D-Ser^8-CsA hapten is illustrated below.

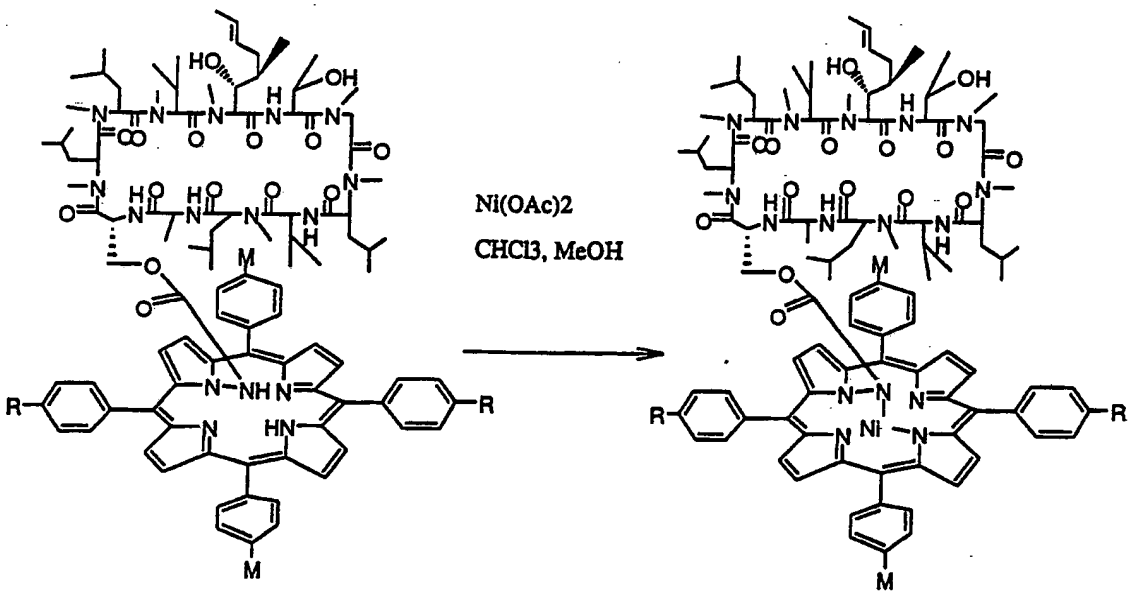

H2N-O-SO2-Ph-Me
CHCl3
(CF3CO)2O
DMF/DMAP
DDQ
CH2Cl2
EtOH, KOH 65°
Diphosgene
DMAP, CH2Cl2, Py
CyA-D-Ser-8

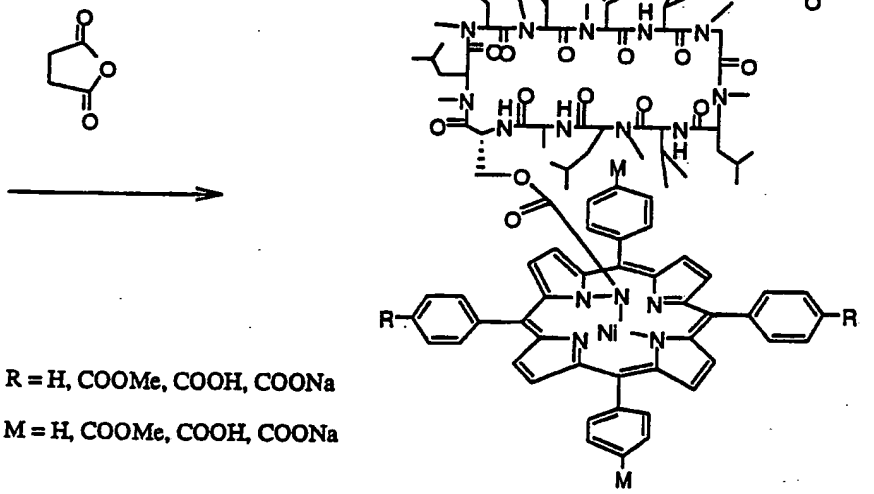

Ni(OAc)2
CHCl3, MeOH
65°


H2, 10% Pd/C
EtOH, HCl
NH2

Other haptens may be produced by similar methods. For example, to produce a hapten in which the residue is further functionalized so that the carrier protein can be attached to it, the following reaction scheme can be adopted:







R = H, COOMe, COOH, COONa

M = H, COOMe, COOH, COONa

The antigen is produced by coupling to the hapten a carrier protein that renders the hapten/carrier protein complex immunogenic. The carrier protein may be covalently bound to the hapten by providing one of the R_1 groups in the form of an amino group; which then forms a bridge between the carrier protein and the hapten. Suitable procedures are disclosed in Richards et al; 1990; Current Research in Photosynthesis, 3, 695-8. The advantage of coupling the carrier protein to the porphyrin portion of the hapten, as opposed to the residue portion, is its general applicability since the residue portion need not bear a further functional group for the attachment of the carrier protein. However if a functional group is present in the residue portion or can be introduced by synthesis, the carrier protein can be attached to it. Other procedures for binding carrier proteins to haptens are disclosed in Harada, A et al; 1990; Chemistry Letters, 917-918 and 1991; Chemistry Letters, 953-956.

The antigens may then be used to immunise mice. The spleen cells of the mice that give a good response are fused with myeloma cells to produce hybridomas. Those hybridomas that secrete monoclonal antibodies specific to the haptens are then selected. These hybridoma techniques are conventional and suitable techniques are disclosed in, for example, Jacob, J., Schultz, P.G., Sugasawara, R and Powell, M; 1987; J. Am. Chem. Soc., 109, 2174 - 2176, Keinan, E. et al; 1990; Pure and Appl. Chem., 62, 2013-2019 and Harada, A et al; 1990; Chemistry Letters, 917-918.

The haptens may also be used to isolate and purify the desired antibodies from the antibodies produced by the various hybridomas. This is a significant advantage since radiolabelled antibodies that bind the desired antibody need not be prepared. This can be done by selecting those antibodies which bind to the haptens; for example by immobilising the haptens in an affinity chromatograph column or radiolabelling them and allowing the antibodies to bind to them. Alternatively, conventional techniques can be used

by raising antibodies against derivatives of the haptens and using these antibodies in radioimmunoassay procedures.

Once the desired antibodies have been isolated, it is possible to determine the DNA sequence coding for the antibody or to determine the amino acid sequence of the antibody. Once this has been done, fragments or protein domains which include the antibody binding regions, can be built. Procedures for doing this are described in WO 90/07861.

The selected and purified antibodies may then be used in reactions to produce the desired product in a manner similar to that described in WO 92/01781. A metalloporphyrin catalyst, which can fit into the pocket of the antibody, is provided. The metalloporphyrin catalyst, the substrate and the antibodies are then combined. An oxygen source is then added under controlled conditions. If desired, the catalyst may be covalently bound to the antibody prior to the reaction as known in the art. Alternatively the catalyst may be added separately from the antibody and allowed to bind to the antibody during the process. The substrate will be able to enter the cavity formed by the antibody and porphyrin only if it is in the correct orientation to the catalyst to produce the desired product.

For example, the D-Ala^{8} of CsA may be converted to D-Ser^{8} by using the following procedure. A catalyst, CsA and antibodies raised to the aminoporphyrin-bridging group-D-Ser^{8}-CsA antigen are then mixed in a suitable solvent. An oxygen source is then added under controlled conditions. CsA with the correct orientation is able to enter the pocket of the antibody and offer the methyl group to be hydroxylated to the metal-oxygen group. The hydroxylated D-Ser^{8}-CsA is then removed. If necessary, the catalyst is removed and regenerated.

In another example, the N-methylgroup of leucine^{4} of CsA may be converted to 4-N-hydromethylleucine by using the following procedure. A catalyst, CsA and antibodies raised

to the aminoporphyrin-bridging group-N-hydromethylleucine^4-CsA antigen are then mixed in a suitable solvent. An oxygen source is then added under controlled conditions. CsA with the correct orientation is able to enter the pocket of the antibody and bond offer the N-methyl group of leucine^4 to the metal-oxygen group of the porphyrin. The hydroxylated N-hydromethylleucine^4-CsA is then removed.

Similar procedures may be used for all other reactions. The source of oxygen atoms may be selected from H_2O_2, iodosobenzene, magnesium monoperoxyphthalate, NaOCl, $KHSO_5$ and the like.

It will be appreciated that substrates that have more than one site that can be hydroxylated, dealkylated, epoxidated, desaturated and the like can be selectively attacked so that only the desired site is altered. Similarly substrates that have prochiral centres that, when reacted, can form diastereomers, can be selectively reacted so that only one diastereomer forms. Similarly single enantiomer products can be produced from substrates that, when ordinarily reacted, form racemic mixtures.

Example 1: Hapten formed from N-Amino-5,10,15,20-Tetraphenyl-21H,23H-Porphyrin-Derivative and N-Hydroxymethylleucine^4-Cyclosporin A

1.1. N-Amino-tetraphenyl-chlorin from Tetraphenyl-porphyrin:

10 g of Tetraphenylporphyrin is dissolved in 500 ml warm chloroform. The solution is then cooled to 20°C and 9.8 g O-mesitylsulfonylhydroxylamine is added to it. The solution is then stirred for 20 hours at room temperature. The green reaction mixture is then heated to 60°C for 1 hour and 2N sodium carbonate with chloroform added. The crystalline residue (10 g) is then separated using column chromatography (500 g Alox N, Activity V). After elution with chloroform, 7.5 g of an adduct is obtained. 680 mg N-amino-tetraphenyl-chlorin is then eluted using a chloroform : Ethanol (ratio

100 : 0.6 to 1.0) mixture.

1.2. N-trifluoroacetylamino-tetraphenyl-chlorin:

631 mg N-amino-tetraphenyl-chlorin is dissolved in 30 ml of absolute dimethyl-formamide and 2 ml pyridine. 122 mg of 4-dimethyl-aminopyridine (1mM) is then added and a solution of 231 mg trifluoro acetic acid anhydride (1.1 mM) in 3 ml methylchloride at 20°C is added dropwise over 5 minutes. The solution is then stirred for 10 minutes. The reaction mixture is then evaporated and the residue is shaken with 2N sodium carbonate and chloroform and then washed once with water. 850 mg of N-trifluoroacetylamino-tetraphenyl-chlorin is obtained.

1.3. N-trifluoroacetylamino-tetraphenyl-porphyrin:

A solution of 850 mg N-trifluoroacetylamino-tetraphenyl-chlorin in 50 ml dichloromethane is mixed with 681 mg (3 mM) DDQ and refluxed for 5 hours. The reaction mixture is then shaken once in 2N sodium carbonate and once in water. The residue (780 mg) is then crystallised out of ethanol (650 mg) and then recrystallised out of an chloroform-ethanol mixture to give 430 mg of N-trifluoroacetyl amino-tetraphenyl-porphyrin.

1.4. N-amino-tetraphenyl-porphyrin:

A suspension of 400 mg of N-trifluoroacetylamino-tetraphenyl-porphyrin in 40 ml of ethanol is mixed with a solution of 0.5 g calcium hydroxide in 10 ml of ethanol. The mixture is then stirred for 30 minutes at 70 to 75°C. The precipitate is then cooled to room temperature, filtered and washed with ethanol. The precipitate is then crystallised out of a chloroform-methanol mixture to give 280 mg of N-amino-tetraphenyl-porphyrin. Mass spectra peaks:- MH^+ 630, $[MH-NH_2]H^+$ 615, and other peaks at 215, 237, 255,

273, 289, 307, 343, 391, 419, 539, 646 and 730.

1.5. Condensation of N-hydroxymethylleucine^4-Cyclosporin A with N-amino-tetraphenyl-porphyrin:

A solution of 44.9 mg of 97% diphosgene is mixed with 2 ml of dichloromethane. The resulting solution is cooled to 0 to 5°C and a solution of 244 mg of N-hydroxymethyl-leucine^4-Cyclosporin A in 5 ml dichloromethane is then added dropwise over 15 minutes. The solution is then stirred at 0°C for 15 minutes and a solution of 126 N-amino-tetraphenyl-porphyrin and 25 mg of 4-dimethylaminopyridine in 1 ml pyridine and 8 ml dichloromethane is added rapidly. The reaction mixture is allowed to react for 2 hours at room temperature and then 2N sodium carbonate and dichloromethane is added. The residue (430 mg) is then purified using column chromatography (65 g Alox basic, activity II, chloroform). 340 mg of the condensation product is obtained.

1.6. Production of a Nickel-complex of the condensation product of step 1.5.:

300 mg of the condensation product is dissolved in a solution of 50 ml chloroform. A solution of 0.8 g of nickel diacetatetetrahydrate in 30 ml methanol is added and the mixture refluxed for an hour. The solution is then reduced and shaken once with chloroform and once with water. The residue is purified using column chromatography (56 g silica gel, acetone : hexane 1:2). 190 mg of the nickel complex is obtained and this is recrystallised using tertiary-butylmethylether and a little petroleum ether.

Example 2: Hapten of N-amino-5,10,15,20-tetraphenyl-21H,23H-porphyrin-derivative and Serine^{8}-Cyclosporin A

2.1. N-amino-tetraphenyl-porphyrin is produced in the same manner as described in Example 1, steps 1.1. to 1.4.

2.2. Condensation of Serine^{8}-Cyclosporin A with N-amino-tetraphenyl-porphyrin:

244 mg of Serine^{3}-cyclosporin A in 5 ml methylchloride is condensed with 126 mg of N-amino-tetraphenyl-porphyrin in a manner totally analogous to that set out in Example 1, step 1.5. 350 mg of the condensation product is obtained.

2.3. Production of the Nickel-complex of the condensation product of step 2.2.:

300 mg of the condensation product is refluxed with a solution of 0.8 g of nickel diacetatetetrahydrate in a manner totally analogous to that set out in example 1, step 1.6. 200 mg of the nickel-complex is obtained and this is recrystallised using tertiary-butylmethylether and a little petroleum ether. Mass spectra peaks:- MH^{+} 1873 and other major peaks at 538, 600, 614, 630, 656.

Example 3: Antigen formed from hapten of example 2

The hapten obtained from step 2.3 is activated as its benzotriazole ester in dimethyl formamide (DMF) using bis[2-oxo-3-oxazolidinyl]phosphinic chloride (BOP)/hydroxy-benzotriazol (HOBt). This is then added to a solution of protein (KLH, BSA or ovalbumin) in 2.5:1 DMSO:borate buffer at pH 8.5. A hapten:protein stoichiometry of 5:1 is used to prevent over-derivatisation and precipitation of the protein. After 4 hours, the reaction mixture is dialyzed against phosphate-buffered saline to remove organic

solvents. A conjugate for each protein is obtained separately.

Example 4 Generation of antibodies

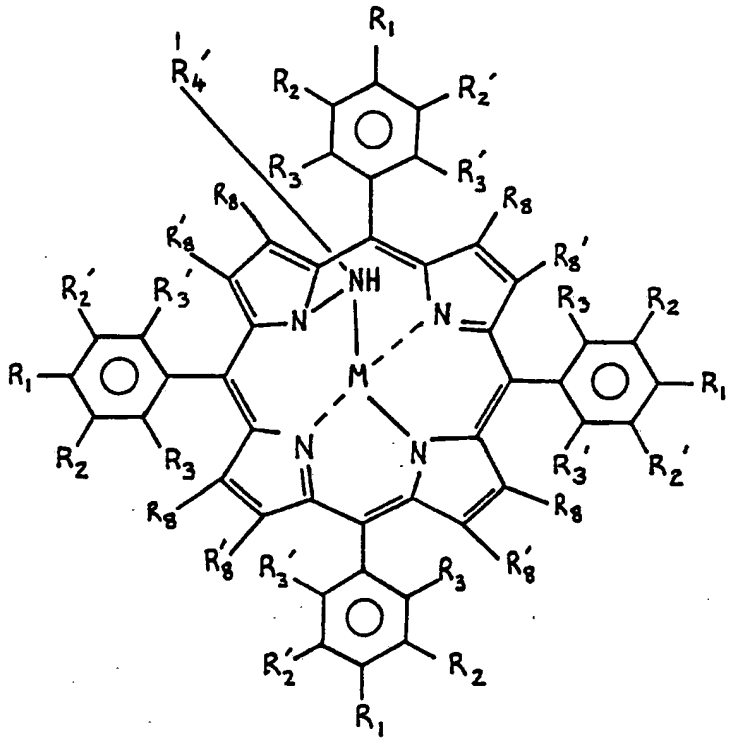
IRCF1 mice are anaesthetized and the peritoneal cavities surgically opened to access the spleen. The surface of the spleen is swabbed with an ethanolic solution containing KLH conjugate obtained from example 3. The mice are immunised in a similar manner on day 22. Serum titres are measured on day 27 by ELISA analysis against free hapten or BSA derivatives absorbed in wells of polystyrene microtitre plates. The antibodies also have partial reactivity with conjugates of the Ser^8-CsA product and a nickel mono-p-amino-tetraphenylporphyrin control. This indicates that antibodies that recognize both the substrate and the catalyst components of the hapten are present in the sera.

BSA and KLH conjugates obtained from example 3 are added to Ribi adjuvant and 6 IRC1 mice are immunised using i.p. injections. On day 14, the mice are boosted with conjugate absorbed on bentonite and the serum sampled on day 17. Three mice showing high serum titres are boosted with further conjugate and are sacrificed on day 31. The spleens from two mice are used in standard fusion protocols using Sp2/0 myeloma cells and PEG. A stable clone which secretes antibody specific for the BSA conjugate is isolated.

The antibodies may be selected and purified using free hapten immobilised in an affinity chromatograph.

Claims

1. A hapten comprising a metalloporphyrin cofactor bound to a residue of a substrate, the hapten mimicking a transition state of a metalloporphyrin catalyst and the substrate in a reaction, characterised in that the metalloporphyrin cofactor is of the formula II:



in which:

each R_1 is independently selected from -H, -F, -Cl, -Br, $-CH_3$, -COOH, $-SO_3H$, $-COO-C_{1-6}$-alkyl, -CH=CH-COOH, $-CH=CH-COO-C_{1-6}$-alkyl, $-SO_3-C_{1-6}$-alkyl, $-NO_2$, phenyl, $-NH_2$, and $-NH-CO-C_{1-6}$-alkyl;

each R_2 and R_2' is independently selected from -H, -F, -Cl, -Br, $-CH_3$, -COOH, $-SO_3H$, $-NO_2$ and phenyl;

each R_3 and R_3' is independently selected from -H, -F, -Cl, -Br, $-CH_3$, $-O-C_{1-6}$-alkyl, $-NO_2$, phenyl, $-NH_2$, and $-NH-CO-C_{1-6}$-alkyl, or

at least one R_3 or R_3' is independently selected from (a) -NH-CO-alkylene-3N-imidazole or -NH-CO-alkylene-

3-pyridine, in which each alkylene has 2 to 4 carbon atoms; and (b) -CO-alkylene-3N-imidazole or -CO-alkylene-3-pyridine, in which each alkylene has 3 or 4 carbon atoms; or

a pair of R_3 and R_3' on opposing phenyl groups jointly form (c) -NH-CO-alkylene-3-pyridyl-5-alkylene-CO-NH- or -CO-alkylene-3-pyridyl-5-alkylene-CO-, in which each alkylene has 2 to 4 carbon atoms;

each R_8 and R_8' is independently -H, -F, -Cl, -Br or -CN;

R_4 is a bridging group connecting the metalloporphyrin catalyst to the residue of the substrate; and

M is a metal ion having a co-ordination number of at least 4; or

an acid addition salt thereof or a sodium, potassium or calcium salt thereof.

2. A hapten according to claim 1 in which the bridging group is of the formula $-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-$ in which R_5 is $-(CO)-$, $-(SO_2)-$ or $-(POOH)-$, R_6 is -O-, -S-, or -(NH)-, and each m, n and p independently is 0 or 1.

3. A hapten according to claim 2 in which the bridging group is $-(SO_2)-O-$ or $-C(=O)-O-$.

4. A hapten according to claim 1 in which each of R_2, R_2', R_3 and R_3' is H.

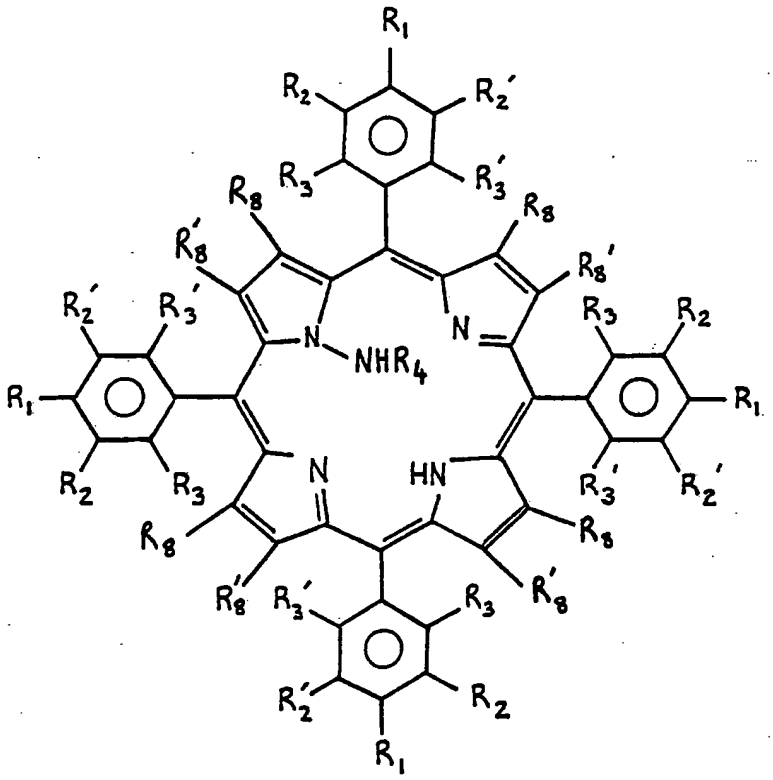
5. A hapten according to claim 4 in which at least one R_1 is $-NH-CO-CH_2-CH_2-COOH$ or $-NH_2$, at least one further R_1 is $-CH=CH-COOH$, -COOH or $-COOCH_3$ and the remainder are H.

6. A hapten according to claim 1 in which the metal ion is Ni^{2+}, Zn^{2+} or Sn^{4+}.

7. A hapten according to claim 1 in which the residue is a cyclosporin residue.

8. A hapten according to claim 7 which is selected from:
the nickel complex of D-Ser^8-CyS-carbamato-N-amino-tetraphenylporphyrin;
the nickel complex of D-Ser^8-CyS-carbamato-N-amino-mono-p-nitro-tetraphenylporphyrin; and
the nickel complex of D-Ser^8-CyS-carbamato-N-amino-mono-p-amino-tetraphenylporphyrin.

9. A compound of formula I:



I

in which:
R_1, R_2, R_2', R_3, R_3', R_8 and R_8' are as defined in claim 1;
R_4 is a) a hydrogen atom; b) a linker group containing a reactive centre or a group through which the compound of Formula I may be bonded to another compound; or c) a removable protecting group; and

acid addition salts of the compound and sodium, potassium and calcium salts of the compound.

10. A compound according to claim 9 in which R_4 is a linker group of the formula $-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-A$ in which R_5 is $-(CO)-$, $-(SO_2)-$ or $-(POOH)-$, R_6 is $-O-$, $-S-$, or $-(NH)-$, m, n and p are each independently 0 or 1 and A is a reactive leaving group or atom, or where p is 1, A may also be hydrogen.

11. A compound according to claim 10 in which m and n are 0 and A is Cl or Br.

12. A compound according to claim 11 in which the linker group is C(=O)-Cl.

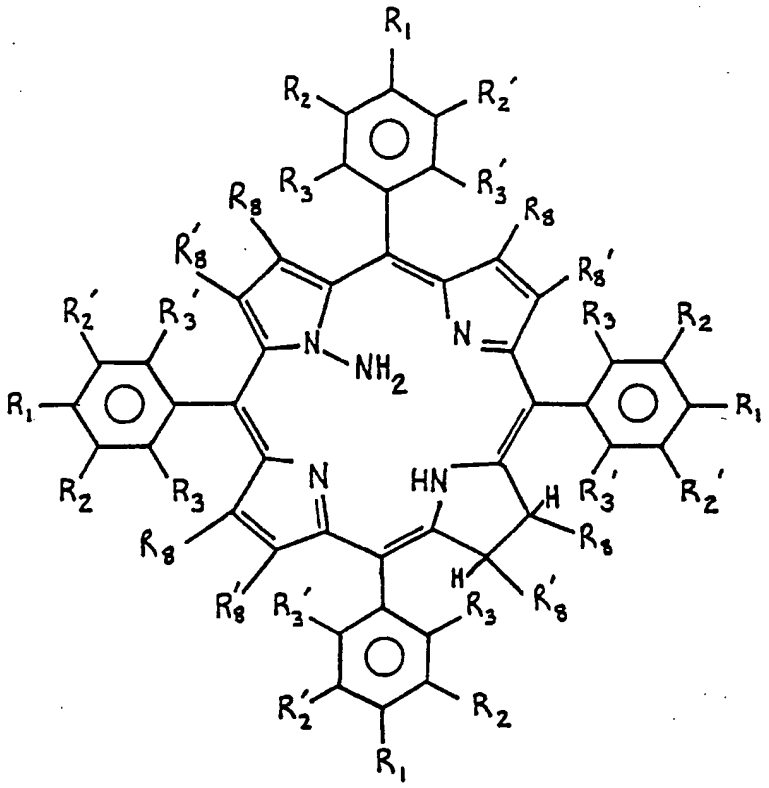
13. A compound according to claim 9 in which R_4 is H.

14. A process for the preparation of a compound of formula I, as defined in claim 9, comprising the steps of:

a) for a compound of formula I in which R_4 is H, deprotecting a compound of formula I in which R_4 is a protecting group;

b) for a compound of formula I in which R_4 is a linker group, reacting a compound of formula I in which R_4 is hydrogen with a precursor of the linker group that contains two reactive atoms or groups, one of which is capable of forming a bond with the $>N-NH_2$ group;

c) for a compound of formula I in which R_4 is a protecting group, i) protecting the $>N-NH_2$ group of a compound of formula III



III

in which R_1, R_2, R_2', R_3, R_3', R_8 and R_8' are as defined above; and ii) oxidizing the compound of formula III to the corresponding compound of formula I.

15. A process according to claim 14 in which, in step a), the protecting group is CF_3CO- and it is removed by alkaline hydrolysis.

16. A process according to claim 14 in which, in step b), the precursor of the linker group is of formula $X-(CH_2)_m-R_5-(CH_2)_n-(R_6)_p-A$ in which X is a reactive leaving group or centre and A, R_5, R_6, m, n, and p are as defined in claim 10.

17. A process according to claim 16 in which the linker group is -CO-Cl- and the precursor is phosgene or diphosgene.

18. A process according to claim 14 in which, in step c), a compound of formula III in which R_4 is hydrogen is reacted with trifluoracetic anhydride in a polar non-aqueous solvent and then oxidized using an oxidizing agent such as 2,3-dichloro-5,6-dicyanobenzoquinone to provide a compound of formula I in which the protecting group is CF_3-CO-.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/02283

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 D 487/22

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	J. Am. Chem. Soc., vol. 111, 1989, Alan W. Schwabacher et al.: "Metalloselective Anti-Porphyrin Monoclonal Antibodies", pp. 2344-2346, see page 2346	1
X	EP, A2, 0305870 (KOLLMORGEN CORPORATION) 8 March 1989, see the whole document	1
P,X	WO, A1, 9201781 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 6 February 1992, see the whole document	1

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18th December 1992	05. 01. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Carolina Gomez Lagerlöf

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Tetrahedron, vol. 35, 1979, H.J. Callot: "Synthesis on N-aminoporphyrins ", pp. 1455-1456 --	9-13
X	Journal of the American Chemical Society, vol. 100, No. 15, July 1978, H.J. Callot et al.: "N-Aminoporphyrins. Preparation and Metal Complexes. Structure of N-Tosylamino-5,10,15, 20-tetraphenylporphinatonickel(II) ", pp. 4733-4741 --	9-13
A	Chemical Abstracts, volume 114, no. 11, 18 March 1991, (Columbus, Ohio, US), Keinan E. et al.: "Towards antibody mediated metallo-porphyrin chemistry", see, abstract 97314p, & Pure Appl. Chem. 1990, 62(10), 2013-2019 --	1-8

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/EP 92/02283

SA 65049

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 02/12/92
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A2- 0305870	08/03/89	AU-D-	2425788	31/03/89
		GB-A-B-	2209340	10/05/89
		JP-A-	1163131	27/06/89
		WO-A-	89/01784	09/03/89
WO-A1- 9201781	06/02/92	AU-D-	8404691	18/02/92

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

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